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A new *Anania* species from Japan, a close relative of *A. crocealis* in Europe (Lepidoptera, Crambidae)

Yutaka YOSHIYASU[1)], Mitsutaka SAKAKIBARA[2)] and Norio HIRAI[3)]

[1)] Entomological Laboratory, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka, 599-8531 Japan

[2)] Entomological Laboratory, National Agriculture and Food Research Organization for Tohoku Region, Simokuriyagawa, Morioka, Iwate, 020-0198 Japan

Abstract A pyraustine, *Anania syneilesidis* n. sp., is described from Japan, with *mt*DNA *COI* sequence and the larval habit. The species is distinct from the closely related species, *A. crocealis* in the western Palaearctic Region, in having different wing markings and genitalic features, in addition to well-developed pinacula on the larval skin. The larva feeds on *Syneilesis palmata* (Asteraceae), which is limited to eastern Asia and a new host plant for *Anania* species. The larvae live gregariously on the host leaves, and cut the proximal parts of the leaf veins before feeding. This performance might be explained as a defensive behavior against poisonous exudates from the host plant. The taxonomic status of the genus *Ebulea* which is treated as a synonym of *Anania* is discussed, with special reference to the genitalic features.

Key words Asteraceae, *Ebulea*, genitalia, *mt*DNA *COI*, Pyraustinae, vein cutting.

Introduction

The genus *Anania* Hübner, 1823 *s. lat.* (Crambidae, Pyraustinae) is mainly distributed in the Holarctic Region and contains 26 species in the Palaearctic Region (Tränkner *et al.*, 2009; Tränkner and Nuss, 2010; Yang *et al.*, 2012). This genus is characterized by having two synapomorphies on the genitalia: an asymmetric sclerite in the phallic apodeme in the male and a digitiform sclerotization of the antrum in the female (Leraut, 2005; Tränkner *et al.*, 2009). The European *Anania crocealis* (Hübner, 1796) had been placed under the genus *Ebulea* Doubleday, 1850 (type species: *Pylaris crocealis* Hübner, 1796) until the generic revision was given by Leraut in 2005.

The 2nd author found an unknown pyraustine species feeding on *Syneilesis palmata* (Thunb.) Maxim. (Asteraceae) in Iwate Pref., northern Honshu, Japan in 2010 and he obtained a total of 10 adult specimens by rearing. Morphological examination of them revealed that the moth species is new to science, much resembling *Anania crocealis*. We describe this new species and give the *mt*DNA *COI* sequence for inferring relationships among the related species of this genus in future study. The Asteraceae are known as host plants for *A. crocealis* and common hosts for the other species of this genus and its allied genera (Hasenfuss, 1960; Goater, 1986). However, *Syneilesis palmata* has not been recorded as a host for the genus. We discuss the genitalic structure and the peculiar larval behavior on the host, comparing it with another pyraustine species. We also discuss the taxonomic status of the genus *Ebulea* on the genitalic characters.

Materials and methods

(1) Morphological observation

Before examining genitalic structures, the removed abdomen was treated in 5% KOH solution in a warm glass tube in hot water for about 30 minutes, then transferred to a petri dish with water for clearing. The samples were transferred to another petri dish with 80 % ethanol, and dissected under a binocular microscope (Leica IC80 HD) and observed in the same dish. The drawings of the genitalia were made under natural conditions in the petri dish before making slide preparations. The photos of morphological traits were taken by camera lucida of the microscope in natural conditions except for the tympanal organ. The organ was mounted on a slide glass with drops of glycerin and covered with cover glass. The larval sample was not available in this work, but the general morphological features were described based on observations in the field and in the rearing room. The terminology of the genitalia follows Yoshiyasu (2011) and Leraut (2005), except for the valva of the male after Munroe (1976).

(2) DNA extraction and PCR amplification

Two adult males collected at Shimokuriyagawa, Morioka, Iwate Pref. (emerged on 2.vii.2010 from larvae collected on 15 & 24.vi.2010, respectively) were used. Total DNA was extracted from the hindlegs of

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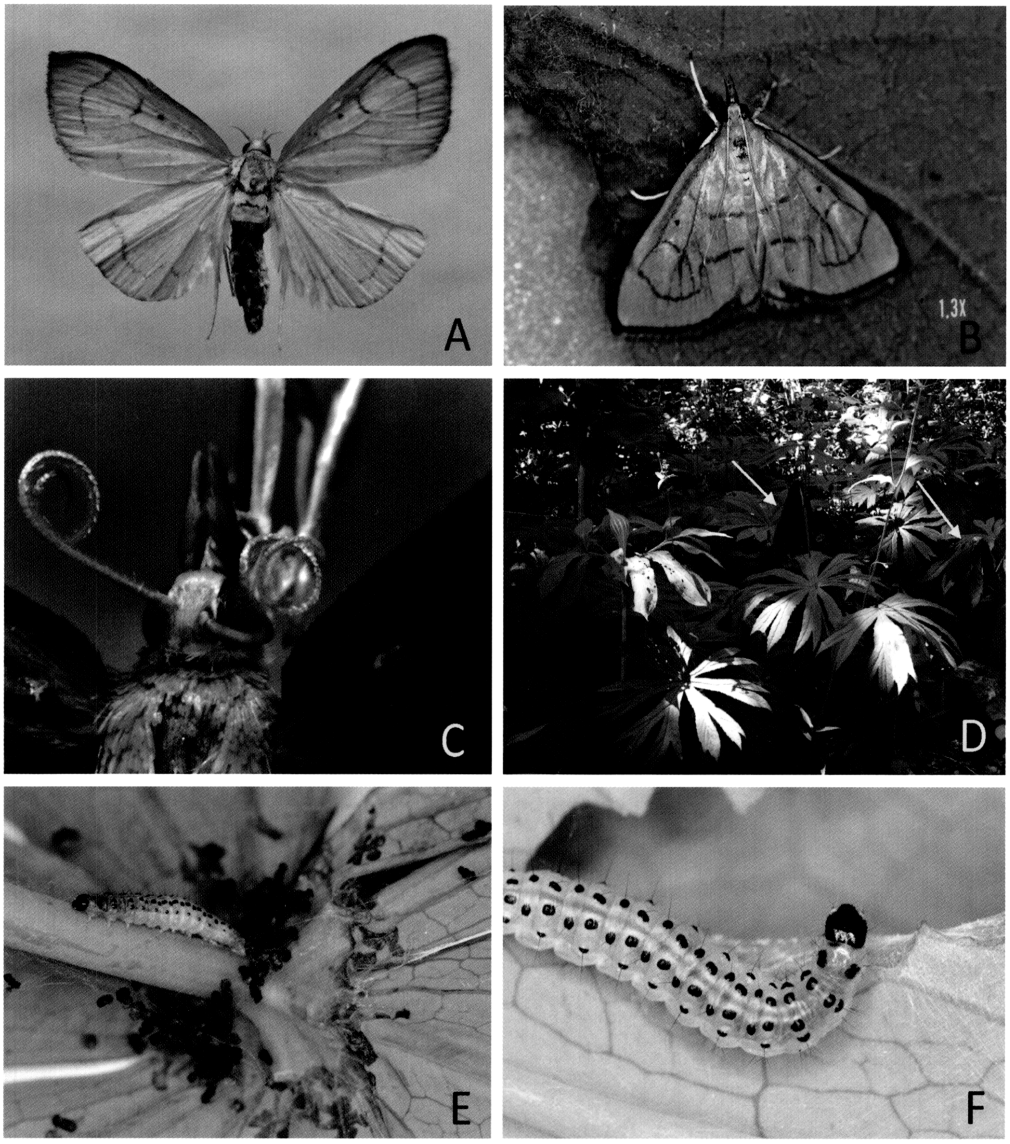

Fig. 1. Adults, larva and feeding habit of *Anania syneilesidis* n. sp. A. adult female, holotype; B. an adult resting on a host leaf; C. adult male, head, dorsal; D. damaged host leaves infested by the larvae (indicated by arrows) in the field; E. vein cutting by the larvae; F. a mature larva on a leaf.

adults using a DNeasy Tissue kit (Qiagen, Valencia, CA, USA). The mitochondrial *COI* gene, which encodes cytochrome oxidase subunit I, was amplified by using the primers LCO 1490 (5'-GGTCAACA AATCATAAAGATATTGG-3') and HCO2198 (5'-T AAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). Other procedures were done following the methods described in Kobayashi *et al*. (2011).

Results

Description

Anania syneilesidis Yoshiyasu & Sakakibara n. sp. (Japanese name: Yaburegasa-nomeiga)

Adult (Figs 1A-C & 2) Forewing length 9.6 – 11.7 mm (n=8).

♂ & ♀ : Head fulvous; frons a little produced anteriorly, vertex with erect, fulvous scales.

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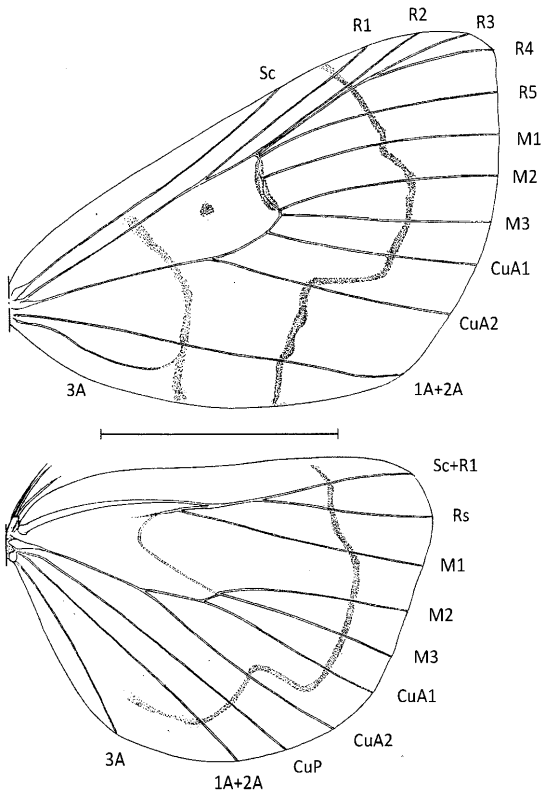

Fig. 2. Wing venation and marking of *Anania syneilesidis* n. sp. (♀). Scale: 5.0 mm.

Labial palpus porrect, almost as long as length of head; the 3rd segment longer than the 2nd one, narrowing to apex, fulvous dorsally and whitish ventrally. Maxillary palpus short, fulvous.Chaetosemata absent. Antenna in male ciliate and dorsally scaled fulvous (Fig.1C), that in female filiform. Foreleg whitish except for anterior portion of femur and tibia slightly suffused with yellow. Mid- and hindleg whitish, with each inner spur 1.5 times as long as the outer one. Forewing (Fig. 2) rather broad, with costal region rather thickly scaled, yellowish to apex; apex rather angulate and termen evenly curved to tornus. Ground color bright yellowish fulvous; antemedial line narrow and rather weak, dark brown from basal 1/3 of costa, curved to 1/3 of posterior margin; a dark brown spot in discoidal cell; discal bar narrow but distinct, dark brown; postmedial line oblique from costal margin to vein M1, then curved outwards to vein CuA2, and strongly retracted proximally, then running sinuously at 2/3 of posterior margin. Hindwing lighter than forewing; antemedial line absent; postmedial line faint, being almost as in forewing, running from 4/5 of costa, then becoming parallel to outer margin and extended to vein CuA2, and retracted proximally, then gradually curbed to 1A+2A and disappearing near posterior margin.

Wing shape and venation (Fig. 2). Wing shape almost similar to *A. crocealis*, but termen behind apex gradually curved to tornus, while in *A. crocealis* termen is slightly excurved behind apex. Wing venation as in *A. crocealis*.

Tympanal organ (Fig. 4A): Tympanal cavity rather wide, secondary venulae weakly developed. Praecinctorium small and rounded, bilobed with fine whitish scales on it.

Male genitalia (Figs 3A-E & 4B-C): Tegumen longer than wide, with a pair of dorsal longitudinal inner ridges. Fenestrulla developed between tegumen and uncus. Vinculum wide laterally, narrowing to rounded saccus laterally. Uncus short, weakly curved downwards, with sparse short setae dorsally. Gnathos (gn) broad extending ventrally where it is fused to proximal end of subscaphium of tuba analis (Fig. 3D). Valva long, with distal half of inner surface densely setose and apex rather narrow and rounded; transtilla broad, weakly developed, with some short setae near apex; clasper well developed, with shaft swollen, having many scale-like setae dorsally and many spines ventrally, its ventral process wide at base, a little elevated, furnished with many small spines apically (Fig. 4B); sacculus broad, without any spine through its length, but with short setae dorsally. Phallus rather short; coecum penis short; suprazonal sheath with a dorsal phallic apodeme strongly sclerotized, asymmetrical and widened distally, with several minute spines along distal margin; vesica

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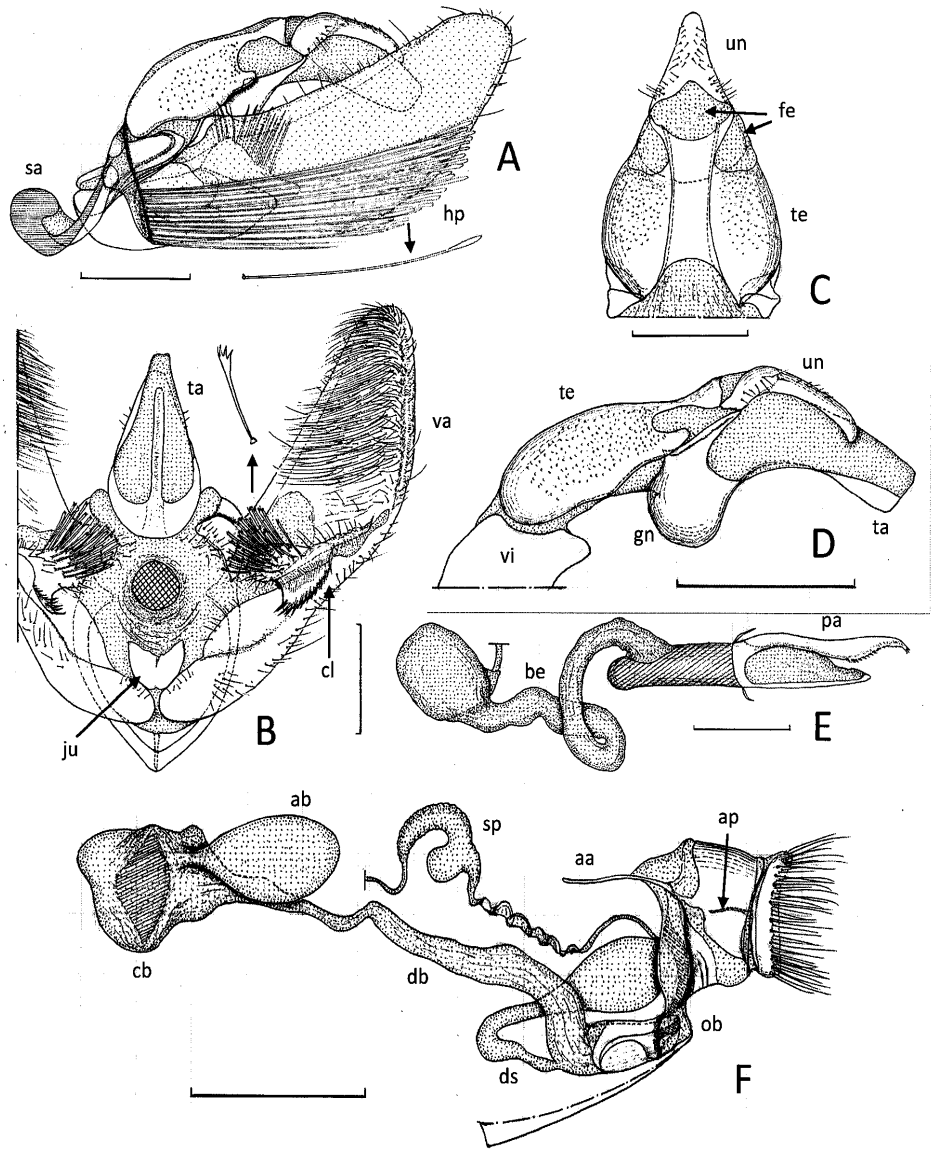

Fig. 3. Male (A-E) and female (F) genitalia of *Anania syneilesidis* n. sp. A. lateral view, with a hair pencil; B, ventral view; C. tegumen & uncus, dorsal view; D. tegumen, uncus & anal tube, lateral view; E. phallus, lateral view. F. female genitalia, lateral view. Abbreviation: aa: apophysis anterioris; ab: appendix bursae; ap: apophysis posterioris; be: burbus ejaculatorius; cb: corpus bursae; cl: clasper; db: ductus bursae; ds: ductus seminalis; fe: fenestrulla; gn: gnathos; hp: hair pencil; ju: juxta; ob: ostium bursae; pa: phallic apodeme; si: signum; sp: spermatheca; ta: tuba analis; te: tegumen; un: uncus; va: valva; vi: vinculum. Scale: 0.5 mm.

without cornutus. Juxta short, heart-shaped.

In addition to the genital structure, the 8th sternum is narrow, and constricted to an anterior area from where a pair of long scale tufts are emitted ventrally and a thick tuft of scales (=hair pencil) from antero-lateral margin of tegumen extending posteriorly (Fig. 3A).

Female genitalia (Figs 3F & 4D-F): Ostium bursae opened on tube-like expansion from antrum and concealed inside cavity forming by sclerotized wall and ventrally supported by a finger-like sclerite (= "digitiform sclerite") which is rounded at apex (Fig. 4D). Ductus bursae long and narrow, gently twisted. Ductus seminalis emitted from anterior margin of antrum; antrum wide, weakly sclerotized. Corpus bursae ovoid (not swollen in unmated female as in Fig. 3F), with a signum almost

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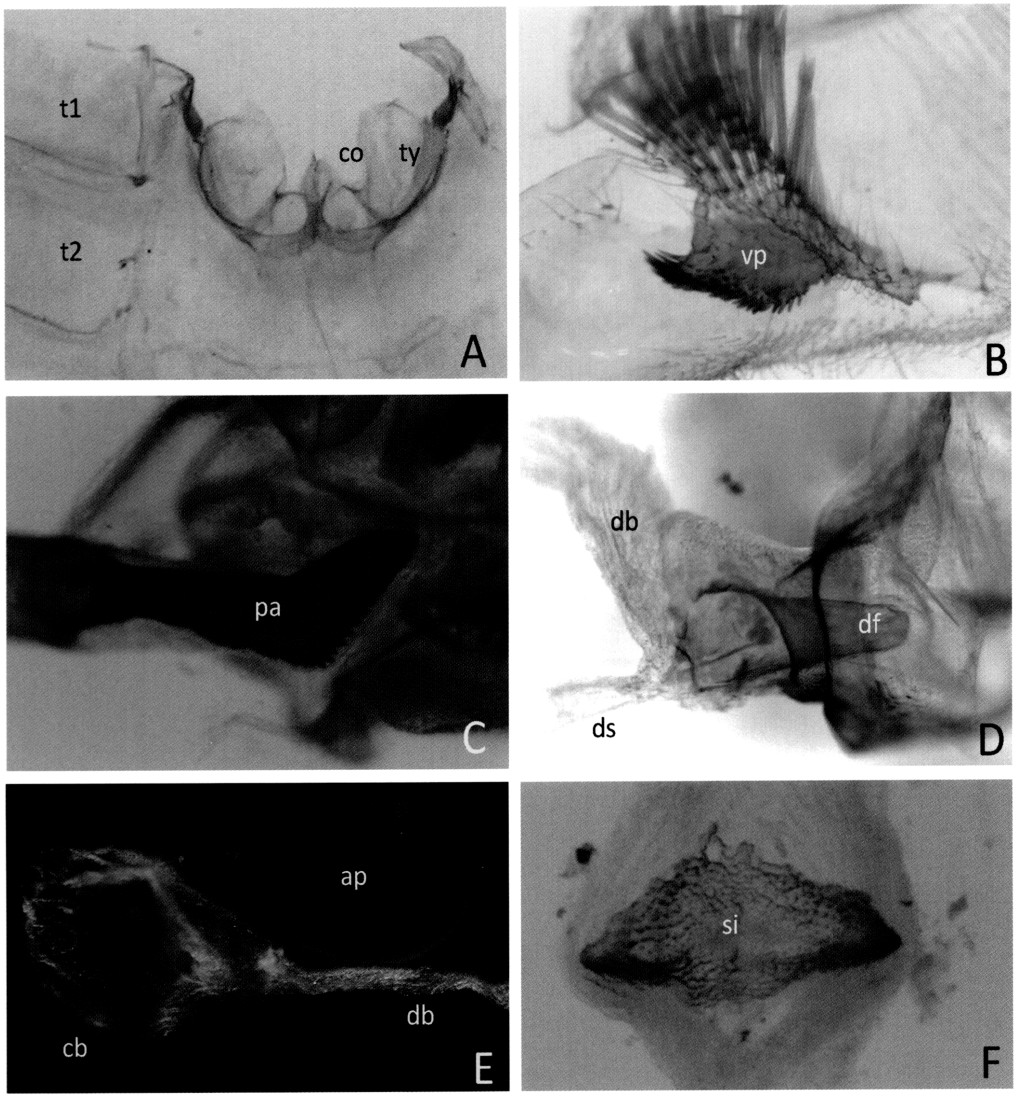

Fig. 4. *Anania syneilesidis* n. sp. A. tympanal organ, ventral view; B. male genitalia, clasper on valva; C. *ditto*, phallic apodeme on phallus, dorsal view; D. female genitalia, ostium bursae, with digitform apodeme, ventral view; E. *ditto*, corpus bursae and appendix bursae; F. *ditto*, signum on corpus bursae. Abbreviation: ab: appendix bursae; ap: apophysis posterioris; cb: corpus bursae; co: conjunctiva; db: ductus bursae; df: digitiform sclerite; ds: ductus seminalis; pa: phallic apodeme; si: signum; sp: spermatheca; t1: 1st abdominal tergum; t2: 2nd abdominal tergum; ty: tympanum; vp: ventral process of clasper.

rhomboidal in shape and coarse on surface (Fig. 4F). Spermatheca with a rather small and rounded lagena laterally. Apophysis anterioris almost straight, about 1/2 the length of 7th sternum; apophysis posterioris short, 1/2 as long as the anterioris. Papilla analis short and wide, with several long setae along posterior margin..

*Mt*DNA *COI* sequence

We obtained partial cytochrome oxidase I (*COI*) sequence (658 bp) and submitted to GenBank (Accession number: AB915324 (DDBJ)).

Type series & depository. Holotype, female (forewing length: 11.1 mm), Shimokuriyagawa, Morioka, Iwate Pref., collected on 15. vi. 2010 (larva), and em. on 2.vii.2010, ex. *Syneilesis palmata*, M. Sakakibara

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leg. (deposited in the National Institute for Agro-Environmental Sciences (NIAES), Tsukuba, Japan). Paratypes: 3♂6♀, same data as in holotype (except for 2♂2♀ collected on 24.vi.2010(larva)) (NIAES except for 1♂& 1♀in Entomological Laboratory, Osaka Prefecture University); 1♂, Hatasho Town, Shiga Pref., 15.vii.1995 (light trap), K. Nakai leg. (deposited in the Lake Biwa Museum).

Distribution. Japan (Honshu: Iwate and Shiga Prefectures).

Etymology. The specific epithet is after the larval host plant, *Syneilesis palmata*.

Mature larva (Fig. 1F). Body length ca. 20 mm; head width ca. 1.6 mm.

Head evenly metallic black, with labrum a little paler. Thorax and abdomen semi translucent as in most pyraustine species, pale milky white, with blackish setae on well developed metallic black pinacula. Prothoracic shield pale brownish white dorsally, blackish postero-laterally. Thoracic legs pale yellow. Abdomen rather stout, with distinct and large pinacula in dorsal and subdorsal regions where D1, D2 and SD setae are present independently. Prolegs with crochets arranged semicircularly and light brown. Anal shield pale yellow.

Host plant. *Syneilesis palmata* (Thunb.) Maxim.

Larval habitat and habit. The host plant of the new species is known as one of the edible wild plants in Tohoku district, and is growing in a rather dusky and wet environment under the canopy of trees in a mountainous region. The larvae were found in a group containing 20 to 30 individuals (last and premature larval instars) under surface of a leaf in the field on 15th and 24th, June in 2010. They usually cut proximal portions of the veins of each leaf underside (Fig. 1E), and then the infested leaf wilted and curved downwards as in Fig. 1D (indicated by arrows). The larvae feed on the wilting leaf almost completely, thinly spinning a web on the surface of the leaf. The mature larvae roll the leaf to make cocoons before pupation.

Remarks. The new species is closely related to the European *Anania crocealis* (Hübner, 1796) in having similar wing markings and genitalic characters, but is distinguished from the latter as follows: 1) the termen behind the apex in the forewing is evenly curved, while in *A. crocealis* it is a little excurved; 2) the postmedial line is narrower, but more distinct and shifted more outwards in the forewing (the distance between the part of the antemedial line and the discal bar and that between portion of vein M1 of postmedial line and discal bar is almost the same)(Fig. 2); 3) the proximal process of the clasper of the male genitalia is broader (Fig. 3); 4) the ostial chamber of the female genitalia is wider than that of *A. crocealis*. In addition to the adult characters, the larva differs from that of *A. crocealis* in having a body with much broader pinacula than those in the latter species, which has narrow pinacula restricted to the base of each seta (Hasenfuss, 1960).

The new species seems to be rare because the second author has found no further larva on the host plant either in the same place in neighboring areas since the first discovery in 2010.

Discussion

(1) Taxonomic status of the genus *Ebulea*

As mentioned in the introduction, *Anania s. lat.* is characterized by having two synapomorphies in the genital characters given in Leraut (2005). The character states are shared by the new species, confirming it as belonging to *Anania s. lat.* Leraut (2005) synonymized the following genera with *Anania*: *Perinephela* Hübner, [1825], *Phlyctaenia* Hübner, 1825, *Ebulea* Doubleday, [1849], *Algedonia* Lederer, 1863, *Coclebotys* Mutuura & Munroe, 1969, *Mutuuraia* Munroe, 1976 and *Proteurrhypara* Mutuura & Munroe, 1969. Following Leraut (2005), Tränkner *et al.* (2009) proposed new synonimies for 4 species belonging to *Opsibotys* Warren, 1890, *Pyrausta* Schrank, 1802, *Perinephela*, *Algedonia* Lederer, 1863, respectively, based on these characters. They also newly synonymized the following 3 genera with *Anania*; i.e. *Pronomis* Mutuura & Munroe, 1968, *Tenerobotys* Munroe & Mutuura, 1971, *Udonomeiga* Mutuura, 1954. Also, Tränkner and Nuss (2010) treated *Ametasia* M. O. Martin, 1986 as a synonym of *Anania*. As a result many species formerly described under the above synonymized genera were transferred to *Anania s. lat.* However, the character states of the two synapomorphies vary among species previously assigned to synonymized genera, from well-developed to poorly developed, or are different in shape. This may be the reason why Leraut (2005) established three subgenera under the genus *Anania s. lat.*, i.e. *Anania* Hübner, 1823, *Eurrhypara* Hübner, [1825] and *Opsibotys* Warren, 1890. In the examination of the structures of the genitalia, we found some important features in two species formerly assingned to *Ebulea* distinct from *Anania s. str.*: 1) the uncus is short and triangular in shape (not long and slender in *Anania s. str.*); 2) the transtillae of both sides are short and not connected at the midvenral line; 3) the gnathos *sensu* Yoshiyasu (2014) is well developed latero-ventrally and rounded; 4) the sacculus of the valva lacks an apical spine in the male genitalia. In the

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female genitalia: 1) the ductus bursae is not so strongly twisted; 2) the appendix bursae is emitted from the middle of the corpus bursae. Considering these rather essential differences, it might be appropriate to treat these two species, formerly assigned to "*Ebulea*", as a distinct subgenus, although Leraut (2005) treated the genus *Ebulea* as the subgenus *Anania*. But this proposal will remain tentative until a more extensive analysis of the genital characters is made and a DNA analysis is completed for the other species of *Anania s. lat.* In relation to the taxonomic treatment of *Anania*, Yamanaka (2013) did not follow Leraut (2005), and he recognized all synonymized genera in the latter paper as being distinct from *Anania s. str.*

(2) Larval habit of *A. syneilesidis* n. sp.

It has been known that the larvae of a pyraustine, *Talanga quadrimaculalis* (Bremer & Grey, 1853) on *Metaplexis japonica* (Apocynaceae) cut the medial vein of the leaf with their mandibles before eating it (Yoshiyasu, 1991). The behavior is well known as "vein cutting" (Dussourd and Eisner, 1987). The Apocynaceae including *Metaplexis* are known to exdate milky and/or usually poisonous fluids if injured. This larval behavior is assumed to minimize the fluids from the plant (Dussourd and Eisner, 1987). The host of the new species, *Syneilesis palmata*, is reported to contain syneilesine, a pyrrolizidine alkaloid (Hikichi and Furuya, 1974). The cutting of proximal veins of leaves by the larvae of this new species (Fig. 1E) may be explained as the same behavior and they actually fed on distal areas of the leaves in the field.

The larvae of some pyraustine species have been reported to be gregarious on their host plants. In Japan, the larvae of *Mutuuraia* (=*Anania*) *terrealis* (Treitschke, 1829) feeding on Aster species (Yoshiyasu, 2011), *Uresiphita prunipennis* (Butler, 1879) on *Sophora flavescens* (Nishio, 2004) and *Udonomeiga* (=*Anania*) *vicinalis* (South, 1901) on *Aralia copdata* and *Angelica ursine* (Komatsu, 2004) are known to be gregarious. The first two species are similar in making web nests covering almost the whole plant leaves and/or shoots, and live inside the nests during the larval to pupal stages. On the other hand, the larvae of *Udonomeiga vicinalis* and the new species do not make nests among leaves similar to those seen in the above two species. Hitherto, there is no report of a gregarious habit either in *Anania crocealis*, feeding on *Inula* and *Pulicaria* species (Asteraceae), or in other species of *Anania* in Europe (Goater, 1986). So it might be explained that the gregarious habit of the larva seen in some *Anania* species in the eastern Palaearctic Region has been developed independently for adaptation to their host plants.

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摘　要

ヨーロッパ産*Anania crocealis* の近縁種の日本からの記載 (鱗翅目, ツトガ科) (吉安　裕・榊原充隆・平井規央)

ノメイガ亜科Pyraustinae の*Anania* 属(広義)はおもに全北区に広く分布し，旧北区ではこれまで26 種(Leraut, 2005; Tränkner & Nuss,2009; Yang *et al.*, 2012)が知られる．第二著者は岩手県盛岡市で，キク科のヤブレガサを加害する日本未記録のノメイガ亜科の幼虫を見出し，生態観察をおこないつつ飼育し成虫を得た．第一著者は，この標本を所検し，ヨーロッパに分布する*Anania crocealis* (Hübner, 1796)にごく近縁の新種と認め，第二著者とともに記載した．また第三著者は，本種の成虫から*mt*DNAの抽出をおこない*COI*領域658塩基対を決定し，GenBankに登録した．本種は，Leraut (2005)で示された広義の*Anania*属の2共有新形質，1) ♂交尾器で挿入器上に遊離した非対象の硬化板(an asymmetric sclerite in the phallic apodeme)をもつこと，2) ♀交尾器にはアントルムの延長にある硬化板(a digitiform sclerotization of antrum)をもつことで，本属の種と認めた．一方，本種およびその近縁種*A. crocealis* (*Ebulea*属のタイプ種)は，*Anania*(狭義)には見られない重要な形態的特徴をもつことを示した．さらに，本種の生息地での野外観察と室内飼育をもとに，幼虫の概略的な色彩と形態ならびに行動を記述し，その摂食行動の意義を述べた．

1. *Anania syneilesidis* Yoshiyasu & Sakakibara n. sp.
ヤブレガサノメイガ(新称)

前翅長：9.6-11.7 mm．頭部は黄褐色でラビアル・パルプスは長く前向し，第3節は細長い．♂の触角は繊毛状で，♀では糸状．成虫は単純な斑紋をもち，前翅はやや黄色を帯びた淡褐色の地色に，細い黒褐色の前横線と後横線のほか，中室内に小黒斑と中室端の黒褐色の棒状斑をもつ；後横線は近縁の*A. crocealis* よりも外側に位置するので，中室端紋と後横線との距離は中室内黒点と中室端紋との間より長い(Fig. 2)(*A. crocealis* では，それらの間はほぼ等距離)．後翅は前翅より淡色で，外横線のみが認められる．♂交尾器のウンクスは短く，長い三角形状で先端は丸い；トランスティラは短く，腹中線まで伸びていない；バルバのクラスパーの腹方突起(Fig. 4Bのvp)は広く先端に多数の微小突起を生じる(*A. crocealis* では，その突起は狭く先端小突起数も少ない)；サックルスは幅広く，基部背方に数本の刺毛をもつほか，腹方には多くの小刺毛が散在する．♀交尾器の交尾口は突出したアントルム後方の先端が丸い指状突起 (Fig.4Dのdf)(*A. crocealis* ではその先端は裁断状)に開口する；コルプス・ブルサエは楕円形で，変形したひし形状のシグナをもつ(Fig. 4Fのsi)．

幼虫の頭部は光沢のある黒色で，胴部は，やや透明な乳白色．胴部のD1，D2　およびSD刺毛群の黒色の刺毛基板が顕著に発達していることで，それが小さく目立たないヨーロッパの*A. crocealis*と区別できる．

2. *Ebulea*属の分類的扱い

本種はLeraut(2005)が挙げた*Anania*属(広義)の2共有新形質をもつが，*Anania*(狭義)とは，以下の交尾器形態で異なるため，現在*Anania*亜属のもとに置かれているヨーロッパの*A. crocealis* とともに別亜属を創設するほうが妥当と考えられるが，今回はその処置を保留した．他の亜属の種や従来*Ebulea*属のもとに記載された種の形態学的検討およびDNA解析の結果をまって，分類学的処置を講じたい．♂交尾器において，1) ウンクスは短く，その基部は幅広く，長い三角形状を呈する(Fig. 3C)；2) 両サイドのトランスティラが腹中線で接することはない(Fig.3B)，3) グナトスが腹方で丸く発達する(Fig.3D)，4) バルバのサックルスの先端に突起をもたない．♀交尾器では，1) ドクツス・ブルサエの捻じれ状構造は弱く，2) アペンデックス・ブルサエはコルプス・ブルサエの後方部ではなく中央部から出る．

3. ヤブレガサノメイガの幼虫の習性

幼虫は東北地区の産地では6月に1枚の葉に20 ～ 30匹が集団で見つけられ，葉の基部の葉脈をかじりとることで，寄主葉を下に垂らし，その中にいて摂食する．この行動は葉脈切り(vein cutting)としてよく知られ，植物体からの有毒なラテックスの分泌を減少させるとされている．本種もほかの種と同じように，葉脈切りをしてヤブレガサに含まれる有毒なピロリジジンアルカロイド(pyrrolizidine alkaloid)であるsyneliesineの分泌を少なくしていると考えられる．老熟幼虫は室内飼育条件下では個別に葉を巻いて繭を紡ぎその中で蛹化・羽化した．

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